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Minireview

Defects of cholesterol biosynthesis

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Abstract Eight distinct inherited disorders have been linked to different enzyme defects in the isoprenoid/cholesterol biosynthetic pathway following the finding of abnormally increased levels of intermediate metabolites in patients and confirmed by the demonstration of disease-causing mutations in genes encoding the implicated enzymes. Patients afflicted with these disorders are characterized by multiple morphogenic and congenital anomalies including internal organ, skeletal and/or skin abnormalities underlining an important role for cholesterol in human embryogenesis and development. The etiology of the underlying pathophysiology may involve multiple affected processes due to lowered cholesterol and/or the elevated, teratogenic levels of the intermediate sterol precursors.

Keywords: Inborn errors; Isoprenoid; Cholesterol; Embryogenesis; Development

1. Introduction

Cholesterol is an important structural component of cellular membranes and myelin, and precursor of oxysterols, steroid hormones and bile acids. Cells acquire cholesterol either by de novo synthesis or via the uptake of low density lipoprotein particles that contain esterified cholesterol. The identification of a number of inherited disorders due to a defect in cholesterol biosynthesis has made clear that cholesterol plays a crucial role in human embryogenesis and development. Currently, eight distinct inherited disorders have been linked to different enzyme defects in the cholesterol biosynthetic pathway after the finding of abnormally increased levels of intermediate metabolites in patients followed by the demonstration of disease-causing mutations in genes encoding the implicated enzymes. Patients afflicted with these disorders are characterized by multiple morphogenic and congenital anomalies including internal organ, skeletal and/or skin abnormalities. Although the genetic causes underlying these defects are known, the etiology of the pathophysiology associated with the defects remains unclear and may involve multiple affected processes due to the lowered cholesterol and/or elevated, teratogenic levels of intermediate sterol precursors.

2. The isoprenoid/cholesterol biosynthetic pathway

Cholesterol is synthesized via the isoprenoid biosynthetic pathway, which produces numerous biomolecules, called isoprenoids, that function in a variety of important cellular processes including cell growth and differentiation, protein glycosylation, signal transduction pathways and mitochondrial electron transport [1]. Isoprenoid biosynthesis starts with the C_2 compound acetyl-CoA, which by means of 6 subsequent enzyme reactions is converted into isopentenyl-PP, the basic C_5 isoprene unit used for synthesis of all subsequent isoprenoids (Fig. 1). The first intermediate committed exclusively to the production of sterol isoprenoids is C_{30} squalene (composed of 6 isoprene units), which after cyclization is converted into C_{30} lanosterol (4,4,14α-trimethylcholesta-8(9),24-dien-3β-ol). For the generation of C_{27} cholesterol from lanosterol, a series of at least 8 different enzyme reactions is required, including one demethylation at C-14, two demethylations at C-4, one isomerization of the $\Delta^{8(9)}$ double bond to Δ^7, three reductions of the Δ^{24}, Δ^{14} and Δ^7 double bonds, and one desaturation between C-5 and C-6 (Fig. 2). Because most of the cholesterogenic enzymes (or enzyme complexes) can handle different sterol intermediates as is also clear from the sterol intermediates that accumulate in the various biosynthesis defects (see below), the sequence of the enzyme steps involved in the conversion of lanosterol into cholesterol may vary dependent on the tissue in which they occur. In general, however, two major routes for cholesterol biosynthesis have been proposed which depend on the timing of reduction of the Δ^{24} double bond and postulate either 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol) or desmosterol (cholesta-5,7-dien-3β-ol) as the ultimate precursor of cholesterol (Fig. 2). The majority of human genes encoding the enzymes involved in post-squalene cholesterol biosynthesis have been identified only recently though the identification and elucidation of the biochemical and molecular basis of inherited defects thereof [2,3].

3. Localization of isoprenoid/cholesterol biosynthesis

Except for HMG-CoA reductase, all enzymes involved in the conversion of acetyl-CoA to farnesyl pyrophosphate, are localized in the cytosol. HMG-CoA reductase and most of the enzymes involved in cholesterol synthesis are localized in the endoplasmic reticulum (ER) [1,4]. In addition, few of the cholesterogenic enzymes are also (i.e. sterol $\Delta^{8(9)}$-isomerase)

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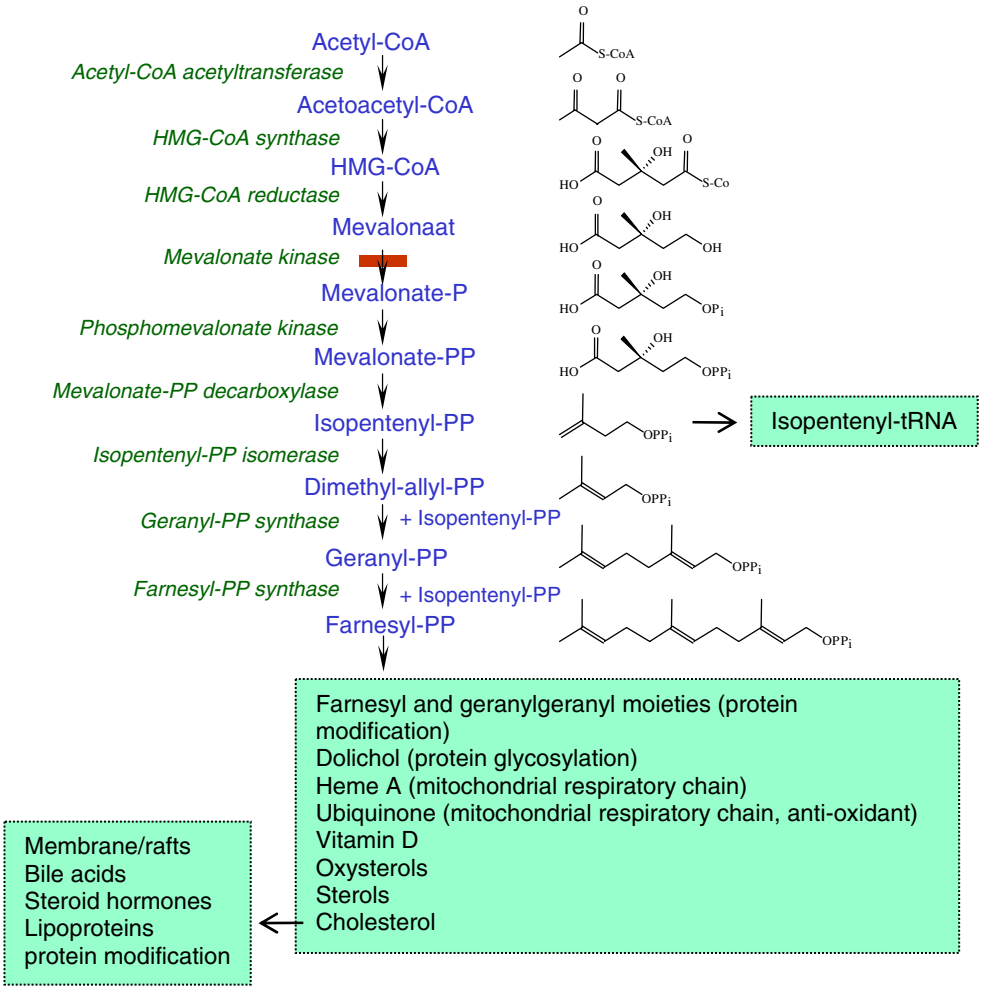

Fig. 1. *Isoprenoid biosynthesis*. The isoprenoid biosynthetic pathway produces numerous biologically active compounds, i.e. isoprenoids, involved in a variety of important cellular processes including the ones indicated in the green boxes. The enzyme mevalonate kinase (red solid bar) is deficient in patients affected with mevalonic aciduria and hyperimmunoglobulinaemia D and periodic fever syndrome.

or primarily (i.e. LBR/sterol Δ^{14}-reductase) found in the nuclear membrane. Since 1985, a series of reports have claimed that many of the enzymes (or the reactions they catalyze) of the presqualene segment of the pathway are partly, mainly, or even exclusively located in peroxisomes, whereas several enzymes involved in cholesterol synthesis were reported to be colocalized in peroxisomes and in the ER. Although since then many conflicting reports on this topic appeared, these studies led to the postulation that peroxisomes would be directly involved in isoprenoid/cholesterol biosynthesis (reviewed in [1,5]). More recent studies have questioned the experimental approaches based upon which this postulation was based [1,5–7]. Indeed, in most cases the claim of a peroxisomal colocalization was based on (a) the finding of only (very) minor amounts of proteins in enriched peroxisomal fractions obtained after subcellular fractionation of rat liver tissue, (b) immunocytochemical localization studies using antisera of undefined specificity, and/or (c) the results of overexpression studies with tagged proteins or (portions of) proteins fused to reporter proteins in cell lines. When combining all available data with emphasis on studies toward the subcellular localization of endogenous proteins under physiological conditions, it should be concluded that there is little, if any, evidence for a direct peroxisomal involvement in the isoprenoid/cholesterol biosynthesis.

4. Defects in isoprenoid/cholesterol biosynthesis

Eight different inherited disorders have been linked to specific enzyme defects in cholesterol biosynthesis after the finding of abnormally increased levels of intermediate metabolites in tissues, body fluids and/or cultured cells of patients followed by the demonstration of disease-causing mutations in genes encoding the implicated enzymes [3].

Two disorders, classic mevalonic aciduria (MIM 251170) and the hyperimmunoglobulinemia D and periodic fever syndrome (HIDS; MIM 260920) are both due to a deficiency of the enzyme mevalonate kinase albeit to different degrees due to specific mutations in the *MVK* gene [8]. Cells of patients with the HIDS presentation invariably have a residual MK enzyme activity of up to 10% of control values, but in cells of patients with the mevalonic aciduria presentation the activity is below detection level. This difference is reflected in the high levels of MK's substrate mevalonic acid in plasma and urine of patients with the mevalonic aciduria presentation and low

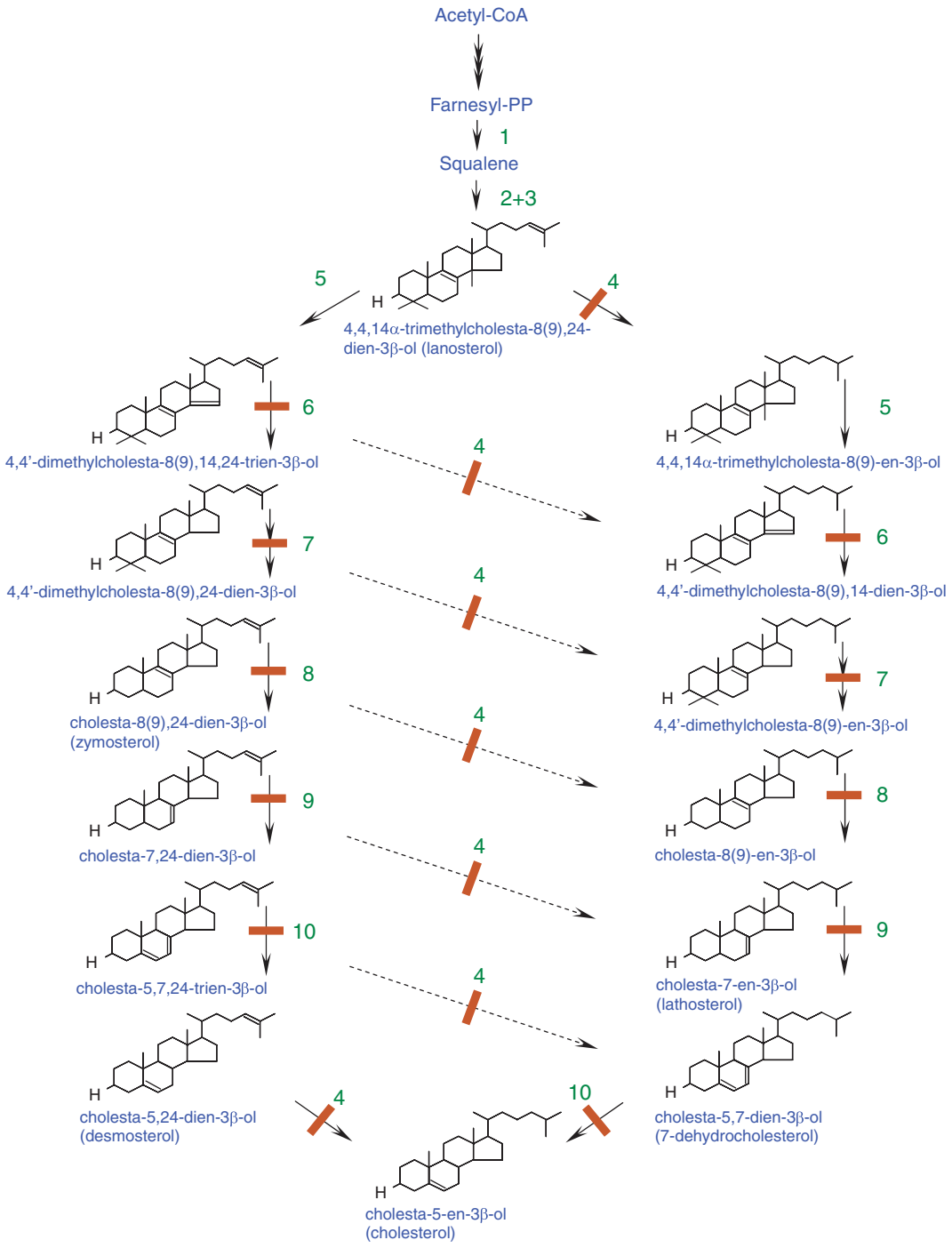

Fig. 2. *Cholesterol biosynthesis*. After cyclization of squalene (composed of six isoprene units) lanosterol is synthesized, which is converted into cholesterol in a series of enzyme reactions requiring one demethylation at C-14, two demethylations at C-4, one isomerization of the $\Delta^{8(9)}$ double bond to Δ^{7}, three reductions of the Δ^{24}, Δ^{14} and Δ^{7} double bonds, and one desaturation between C-5 and C-6. Indicated are the two major routes involved in cholesterol synthesis. In this segment of the pathway six inherited disorders have been linked to specific enzyme deficiencies (indicated by red solid bars). Numbering of the enzymes: 1, squalene synthase; 2, squalene epoxidase; 3, 2,3-oxidosqualene sterol cyclase; 4, sterol Δ^{24}-reductase (desmosterolosis); 5, sterol C-14 demethylase; 6, sterol Δ^{14}-reductase (HEM dysplasia); 7, sterol C-4 demethylase complex (including a 3β-hydroxysteroid dehydrogenase defective in CHILD syndrome); 8, sterol Δ^{8}-Δ^{7} isomerase (CDPX2); 9, sterol Δ^{5}-desaturase (lathosterolosis); 10, sterol Δ^{7}-reductase (SLO syndrome).

to moderate levels of mevalonic acid in patients with the HIDS presentation. Although originally defined as two distinct syndromes, they are now recognized as the severe and mild presentation of one defect, named mevalonate kinase deficiency. Because mevalonate kinase functions early in the pathway, the synthesis of all isoprenoids is affected, although even in severely affected patients cholesterol levels are still low normal (reviewed in [8]). Patients with mevalonate kinase deficiency characteristically present with recurrent episodes of high fever associated with abdominal pain, vomiting and diarrhoea,

(cervical) lymphadenopathy, hepatosplenomegaly, arthralgia and skin rash, and, in severe cases, may present with additional symptoms such as mental retardation, failure to thrive, ataxia, cerebellar atrophy, hypotonia and dysmorphic features [9,10].

The remaining six enzyme defects exclusively affect sterol synthesis and involve four autosomal recessive and two X-linked dominant inherited syndromes. In general, patients afflicted with these defects present with multiple congenital, developmental and morphogenic anomalies, including internal organ, skeletal and/or skin abnormalities, and/or a marked delay in psychomotor development underlining cholesterol's pivotal role in human embryogenesis and development.

The most common defect of cholesterol biosynthesis is autosomal recessive Smith–Lemli–Opitz syndrome (SLOS; MIM 270400), a multiple malformation syndrome caused by 3β-hydroxysterol Δ^7-reductase deficiency (mutations in the *DHCR7* gene at 11q13). The deficiency results in low cholesterol and elevated levels of 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol) and its isomer 8-dehydrocholesterol (cholesta-5,8(9)-dien-3β-ol) in plasma, cells and tissues from these patients. Patients with SLOS often present with a large and variable spectrum of morphogenic and congenital anomalies, including dysmorphic craniofacial features, microcephaly, multiple internal organ, limb/skeletal, and urigenital malformations, (intrauterine) growth and mental retardation, and behavioral problems [11].

Desmosterolosis (MIM 602398) and lathosterolosis (MIM 607330) are two autosomal recessive cholesterol biosynthesis defects with a rather similar clinical presentation as SLOS, but for each of which so far only two patients have been reported. Desmosterolosis is caused by mutations in the *DHCR24* gene at 1p31.1-p33 causing a deficiency of 3β-hydroxysterol Δ^{24}-reductase resulting in low cholesterol and elevated levels of desmosterol [12]. Lathosterolosis is caused by mutations in the *SC5D* gene (at 11q23.3) causing a deficiency of 3β-hydroxysterol Δ^5-desaturase, resulting in low cholesterol and elevated levels of lathosterol in plasma, tissue and cultured cells [13,14].

Autosomal recessive Greenberg skeletal dysplasia (MIM 215140), also known as HEM skeletal dysplasia, is rare severe cholesterol biosynthesis defect that leads to early in utero lethality. Affected fetuses are characterized by fetal hydrops, short-limb dwarfism with an unusual 'moth-eaten' appearance of the shortened long bones, bizarre ectopic ossification centers and disorganization of chondro-osseous histology. The defect is caused by mutations in the *LBR* gene at 1q42 causing a deficiency of the lamin B receptor [15], which functions as the 3β-hydroxysterol Δ^{14}-reductase, resulting in elevated levels of cholesta-8,14-dien-3β-ol and cholesta-8,14,24-trien-3β-ol in tissues or cultured cells. Heterozygosity for a mutation in the lamin B receptor gene results in Pelger–Huet anomaly (MIM 169400), a rare benign autosomal dominant disorder of leukocyte development characterized by hypolobulated nuclei and abnormal chromatin structure in granulocytes of heterozygous individuals [16].

The two X-linked dominant inherited disorders Conradi-Hünermann–Happle syndrome (CDPX2; MIM 302960) [23] and CHILD syndrome (MIM 308050) are caused by deficiencies of sterol Δ^8-Δ^7 isomerase (mutations in the *EBP* gene at Xp11.22-23) [17,18] and/or a sterol C-4 demethylase (mutations in the *NSDHL* gene at Xq28) [19], respectively. Most CDPX2 patients have normal cholesterol levels but elevated plasma levels of cholesta-8(9)-en-3β-ol and, to a lesser extent, cholesta-s5,8(9)-dien-3β-ol (8-dehydrocholesterol). Patients with CHILD syndrome may accumulate 4-methyl sterols and to a lesser extent 4,4′-dimethyl sterols and 4-carboxy sterols, but these are usually not detected. Patients with CDPX2 or CHILD syndrome, most of whom are female, clinically present with skeletal and skin abnormalities, including chondrodysplasia punctata (epiphysic stippling), shortening of long bones, ichtyosis and hyperkeratosis. In CDPX2, the expression of these abnormalities is bilateral and asymmetric, while in CHILD syndrome the expression is unilateral and most frequently affecting the right side of the body [20].

Although accumulation of lanosterol has been described in a few patients diagnosed with Antley–Bixler syndrome, suggesting a defect of lanosterol C14-demethylase, no mutations in *CYP51*, the gene encoding lanosterol C14-demethylase were identified. In stead it appeared that the reduced activity of this enzyme (as well as enzymes of steroidogenesis) results from mutations in the *POR* gene encoding cytochrome P450 oxidoreductase [21].

5. Regulation of isoprenoid/cholesterol biosynthesis

The rate-controlling step of the isoprenoid/cholesterol biosynthesis pathway is catalysed by HMG-CoA reductase, the enzyme activity of which is controlled by multiple regulatory mechanisms. These include regulation at gene transcriptional level, efficiency of mRNA translation, rate of protein degradation and modulation of enzymatic activity [1].

Transcriptional regulation of the HMG-CoA reductase gene occurs via feedback regulation in response to sterol levels and is mediated by two members of the sterol regulatory element binding protein (SREBP) family, i.e. SREBP2 and SREBP-1a. SREBPs are transcription factors synthesized as large inactive precursors located in the ER membrane where they are tightly associated with the SREBP-cleavage-activating protein (SCAP) escort protein [22,23]. At high sterol concentrations, SCAP interacts strongly with one of two highly similar Insig proteins, which causes the SREBP–SCAP complex to retain within the ER compartment. At low sterol concentrations, the interaction is weakened allowing the SREBP–SCAP complex to transfer to the Golgi apparatus, where cleavage between the N-terminal DNA binding/transcription activation domain and the C-terminal transmembrane/regulatory domain of SREBP occurs, mediated by the subsequent action of two Golgi-bound proteases S1P and S2P. The N-terminal domain is then translocated to the nucleus where it transcriptionally activates target genes containing sterol regulatory element sequences, including the HMG-CoA reductase gene, the LDL receptor gene as well as many other genes involved in isoprenoid/cholesterol biosynthesis [22,23]. The sterol-dependent translocation of the SREBP–SCAP complex to the Golgi apparatus is dependent on a functional sterol-sensing domain in the SCAP protein, which binds directly to cholesterol, causing a conformational change of SCAP enabling the interaction with Insig [24]. This sterol-sensing domain is a conserved membrane-bound region encompassing 5 predicted membrane-spanning helices with short intervening loops, which has been found in several proteins that all have some relation to sterol homeostasis. Although 25-hydroxycholesterol does not directly bind the sterol-sensing domain of SCAP, it also induces

a conformational change of SCAP leading to the interaction with Insig [24].

HMG-CoA reductase also contains a sterol-sensing domain, which at high sterol concentrations binds sterols and one of the Insig proteins. In this case the binding leads to ubiquitination and proteasomal degradation of HMG-CoA reductase [25]. The ubiquitination is specifically stimulated by lanosterol, the first sterol intermediate in cholesterol biosynthesis, oxygenated derivatives thereof or oxysterols such as 25-hydroxysterols, whereas cholesterol has no stimulating effect [26]. This indicates that hydroxyl groups in the side chain and methyl groups at the C4 and/or C14 position are key determinants for the sterol-regulated degradation of HMG-CoA reductase. The sterol-accelerated degradation of HMG-CoA reductase is enhanced by non-sterol isoprenoids, including derivatives from farnesyl pyrophosphate (e.g. farnesol) and geranylgeranyl pyrophosphate [25,27].

Interestingly, lanosterol does not bind the sterol-sensing domain of SCAP and, accordingly, does not suppress the processing of SREBP [26]. Thus, oxysterols (derived by conversion of cholesterol) may downregulate HMG-CoA reductase by increased ubiquitination-mediated degradation as well as suppression of HMG-CoA reductase gene transcription by blocking the exit of the SREBP–SCAP complex from the ER, whereas lanosterol selectively increases the HMG-CoA reductase degradation rate, and cholesterol selectively blocks the translocation of SREBP–SCAP. This confirms earlier data suggesting that lanosterol is the key intermediate and its methylation the rate-limiting step in sterol synthesis. Moreover, the above discussed mechanisms imply that while lanosterol inhibits its own synthesis through downregulation of HMG-CoA reductase, its overaccumulation is prevented because the transcription of the genes encoding the subsequent cholesterol synthetic enzymes remains unaffected, owing to lanosterol's inability to suppress SREBP processing [26].

Expression of HMG-CoA reductase is also regulated at the translational level, where the translation rate of HMG-CoA reductase mRNA is dictated by the cells' demand for non-sterol isoprenoids. When mevalonate production by HMG-CoA reductase is blocked by statins, HMG-CoA reductase mRNA is translated efficiently even in the presence of sterols, but when also the non-sterol requirements are fulfilled by the addition of mevalonate, the translation rate reduces fivefold [28].

Finally, the catalytic activity of HMG-CoA reductase can be downregulated via phosphorylation by the AMP-dependent kinase under influence of the cellular energy state (ATP/AMP ratio) [29].

6. Regulation of isoprenoid/cholesterol biosynthesis in cholesterol biosynthesis defects

As already indicated, patients with mevalonate kinase deficiency in general have normal or low normal levels of cholesterol implying that despite the decreased mevalonate kinase activities these patients still are capable of generating sufficient isoprenoid end products. In part this is reflected in the increased activity of HMG-CoA reductase in cells of these patients which compensates for the decreased flux [8]. However, whereas the flux through the pathway may be sufficient under normal conditions it has been postulated that the pathophysiology associated with this defect is due to the inability to respond rapidly and adequately to an instant further decrease in the activity of mevalonate kinase leading to a (temporary) shortage of a non-sterol-isoprenoid dependent factor required to repress a massive inflammatory response [8].

The female patients affected with one of the two X-linked dominant defects also usually have normal to low normal cholesterol and total sterol levels, which can be explained by the X-chromosome inactivation pattern, which gives rise to cells expressing the mutant allele as well as cells expressing the normal allele.

Most of the remaining autosomal recessive cholesterol biosynthesis defects are not only associated with low cholesterol but also with low total sterol concentrations, indicating a disturbed regulation of sterol biosynthesis most probably caused by the accumulating sterol intermediate.

One possible scenario to explain the decreased total sterol levels is that the sterol intermediates inhibit SREBP processing, which would result in a lowered transcription of genes involved in sterol synthesis. However, although desmosterol was shown to bind to the sterol sensing domain of SCAP in vitro and induces the same conformational change of SCAP as cholesterol [30], the sterol intermediates lanosterol, zymosterol, zymostenol, lathosterol, 7-dehydrocholesterol, and also desmosterol do not inhibit SREBP processing in intact cells [26] and thus do not exert a more potent feedback regulation via SREBP. This is also confirmed by the mRNA levels of the SREBP-responsive HMG-CoA reductase and LDL-receptor genes in cells from *dhcr7*$^{-/-}$ mice (i.e. a mouse model for SLOS), which are similar to the mRNA levels in control mice [31].

Another scenario is that the sterol intermediates downregulate sterol synthesis via a posttranslational regulatory mechanism. Both in cells of SLOS patients and in the *dhcr7*$^{-/-}$ mouse, 7-dehydrocholesterol was found to be a potent inhibitor of HMG-CoA reductase at the protein but not the transcriptional level reducing the rates of sterol biosynthesis [31,32]. Although in one in vitro study, it was shown that 7-dehydrocholesterol, or a derivative thereof, accelerates the degradation of HMG-CoA reductase [31], another study reported that 7-dehydrocholesterol as well zymosterol, zymostenol, lathosterol and desmosterol did not promote ubiquitination and degradation of HMG-CoA reductase [26].

7. Cholesterol and embryogenesis

The overall clinical presentation of patients with a cholesterol biosynthesis defect is consistent with an important role for cholesterol in human embryogenesis and development. This has been attributed to cholesterol's role in hedgehog signaling [33,34]. The hedgehog proteins, including Sonic, Indian and Dessert hedgehog, are secreted signaling molecules (morphogens), which have been implicated in different embryonic patterning processes, many of which are disturbed in patients with one of the cholesterol biosynthesis defects [33,34].

The hedgehog proteins are subject to an autocatalytic cleavage process mediated by their C-terminal domain, during which cholesterol becomes covalently attached to the N-terminal signaling domain. The cholesterol adduction enables the insertion of the N-terminal domain in the plasma membrane, where it interacts with its receptor Patched. This interaction may be promoted by the cholesterol moiety, because Patched

possesses a sterol-sensing domain, although interaction with Patched has also been observed in the absence of cholesterol. The interaction with hedgehog alters Patched's interaction with another membrane protein called Smoothened and releases Smoothened's repression of the hedgehog protein signal transduction pathways, resulting in transcriptional activation of genes involved in embryonic patterning and morphogenesis [33,34].

It is assumed that perturbations of the hedgehog protein signal transduction pathways are responsible for many of the congenital abnormalities associated with the cholesterol biosynthesis defects. Indeed, in contrast to cholesterol, 7-dehydrocholesterol fails to stimulate the Sonic hedgehog signaling pathway [33]. Initially it was hypothesized that either the low levels of cholesterol, the presence of aberrant sterols or both would disrupt the autocatalytic processing of the hedgehog proteins. However, Sonic hedgehog is also normally processed with 7-dehydrocholesterol and desmosterol in an in vitro assay as well as upon expression in embryonic fibroblasts derived from a Smith–Lemli–Opitz syndrome or a lathosterolosis mouse model [35]. Based on these findings and because Patched contains a sterol-sensing domain, it then was hypothesized that impaired hedgehog signaling may be due to changes in the intracellular sterol concentrations. Indeed, it was found that the response to Sonic hedgehog in the mouse SLO and lathosterolosis embryonic fibroblasts is impaired at the level of Smoothened and caused by decreased sterol levels rather than by the accumulation of abnormal sterol intermediates [35]. Yet, it can still not be excluded that the interaction of the sterol-modified hedgehog signaling domain with Patched is less efficient with one of the accumulating sterol precursors than with cholesterol. An interesting finding in this respect is the association of the cholesterol-modified hedgehog signaling domain, Patched and Smoothened with raft lipid microdomains [36]. In addition to the possibility that the efficiency of signaling might depend on the localization of the hedgehog signaling domain to rafts, the association may also be required for the spatially restricted subcellular and tissue distribution of the domain. The formation of such lipid rafts is highly dependent on the structure of the sterol component, which promotes the packing with lipids having saturated acyl chains (e.g. sphingolipids), and 7-dehydrocholesterol, the accumulating sterol in Smith–Lemli–Opitz syndrome, is significantly more strongly lipid raft-promoting than cholesterol [37]. Thus, both the overall sterol levels and the composition of these may have effects on the formation and/or functioning of lipid rafts and consequently on that of its protein components [38]. Hence, it may well be possible that in patients with a cholesterol biosynthesis defect, the formation of rafts and/or the localization of the sterol-modified hedgehog signaling domains, Patched and/or Smoothened to these rafts is disturbed leading to impaired hedgehog signaling.

8. (Maternal) Cholesterol in embryogenesis and development

Most clinical features associated with the cholesterol biosynthesis defects are of early fetal and embryogenic origin. The growing fetus has a high demand for cholesterol, which during early pregnancy is maternally derived whereas during late pregnancy the fetus entirely relies on its own high cholesterol biosynthesis capacity [39]. A strong indication for transfer of maternal cholesterol to the fetus comes from the observation that fetuses with SLOS, who have two null mutations in the *DHCR7* gene and thus cannot synthesize cholesterol, still have low amounts of cholesterol in their tissues and blood. In fact, the amount of maternal (LDL) cholesterol that is transported to the fetus across the placenta may be an important denominator for the severity of the pathology arising during early stages of embryonic development in fetuses with a cholesterol biosynthesis defect. Because these fetuses have low cholesterol synthesis rates, the fetal cholesterol concentrations are more dependent on and in fact have been shown to correlate with maternal cholesterol concentrations as is the clinical severity in these fetuses [39,40]. Furthermore, the clinical presentation of patients with SLOS is more severe in patients whose mothers carry the apo ε2 allele than in patients whose mothers do not carry this allele [41]. Apo ε2-containing LDL cholesterol binds less effective to the LDL receptor, decreasing the transport efficiency of maternal LDL cholesterol to the fetus.

The contribution of maternal cholesterol to fetal cholesterol homeostasis is more prominent in rodents [39] and appears essential for early embryonic development, as also evident from the lethal fetal phenotype of mice with defects in placental cholesterol transport [42]. Moreover, although in general they display similar severe symptoms as their human counterparts, mice with defects in the postsqualene part of cholesterol biosynthesis pathway often survive to term [14,31,43], whereas mice with a defect in the pre-squalene part do not [44]. A striking example of this are the two desmosterolosis mouse models, which both were generated by knocking-out the *dhcr24* gene. In contrast to the few patients known with this defect, one mouse model had a relatively mild phenotype [45] whereas the other mouse model in another genetic background showed a lethal phenotype [46]. Sterol analysis of plasma and liver of 3-months-old *dhcr24*$^{-/-}$ mice with the mild phenotype revealed that desmosterol accounted for >90% of all the sterols. However, sterol analysis of *dhcr24*$^{-/-}$ embryos showed that 60% and 30% of the total sterols comprised of cholesterol at gestation days 11.5 and 17.5, respectively, which can only be of maternal origin, because the fetus is incapable of converting desmosterol into cholesterol [45]. Whereas the maternally derived cholesterol may allow embryonic development of the *dhcr24*$^{-/-}$ fetuses per se, the relative mild phenotype and the occurrence of rather normal total sterol plasma levels of the 3-months-old *dhrc*24$^{-/-}$ mice indicates that desmosterol may substitute for cholesterol in selected processes, including the maintenance of sterol homeostasis via SREBP as discussed above. The lethal phenotype of the second mouse model has been attributed to the marked difference in cholesterol absorption in mice with different genetic backgrounds [46]. The lower cholesterol absorption in the latter genetic background may not allow for sufficient maternal cholesterol transport to the fetus.

9. Conclusions

The complicated clinical phenotype of patients affected with one of the inherited defects of cholesterol biosynthesis, including the multiple affected developmental processes, underlines the important roles of cholesterol and its biosynthetic pathway in a large variety of cellular processes that have become apparent in the past decades. The etiology underlying the

pathophysiology associated with these defects may involve multiple affected processes, ranging from defective regulation of the isoprenoid/cholesterol biosynthesis pathway to defects in developmental processes, which depend on specific isoprenoids/sterols produced by the pathway. These processes are most probably defective as a consequence of the combined effect of low cholesterol levels, elevated (teratogenic) levels of sterol intermediates and a variable ability of these sterol intermediates to functionally substitute for cholesterol in cholesterol-dependent pathways.

Acknowledgements: Owing to the restriction in number of references to be cited, many interesting references are not referred to. Where possible, recent reviews have been cited, which discuss various aspects in more detail and include additional references.

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